A STUDY ON CARDIAC TROPONIN T IN EARLY DIAGNOSIS OF MYOCARDIAL INJURY DUE TO PERINATAL ASPHYXIA & ITS COMPARISION WITH OTHER MODALITIES

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CERTIFICATE

This is to certify that the dissertation titled "**A STUDY ON CARDIAC TROPONIN T (CARD TEST) IN EARLY DIAGNOSIS OF MYOCARDIAL INJURY IN PERINATAL ASPHYXIA & ITS COMPARISION WITH OTHER MODALITIES**" is a bonafide work done by **DR.A.GEETHANJALI** in **M.D BRANCH VII PAEDIATRICS** at Government Mohan Kumaramangalam Medical College Hospital, Salem-636030, to be submitted to the Tamil Nadu Dr. M.G.R Medical University, in partial fulfillment of the University Rules and Regulation for the award of M.D BRANCH VII PAEDIATRICS under my supervision and guidance, during the academic period from May 2010 to April 2013.

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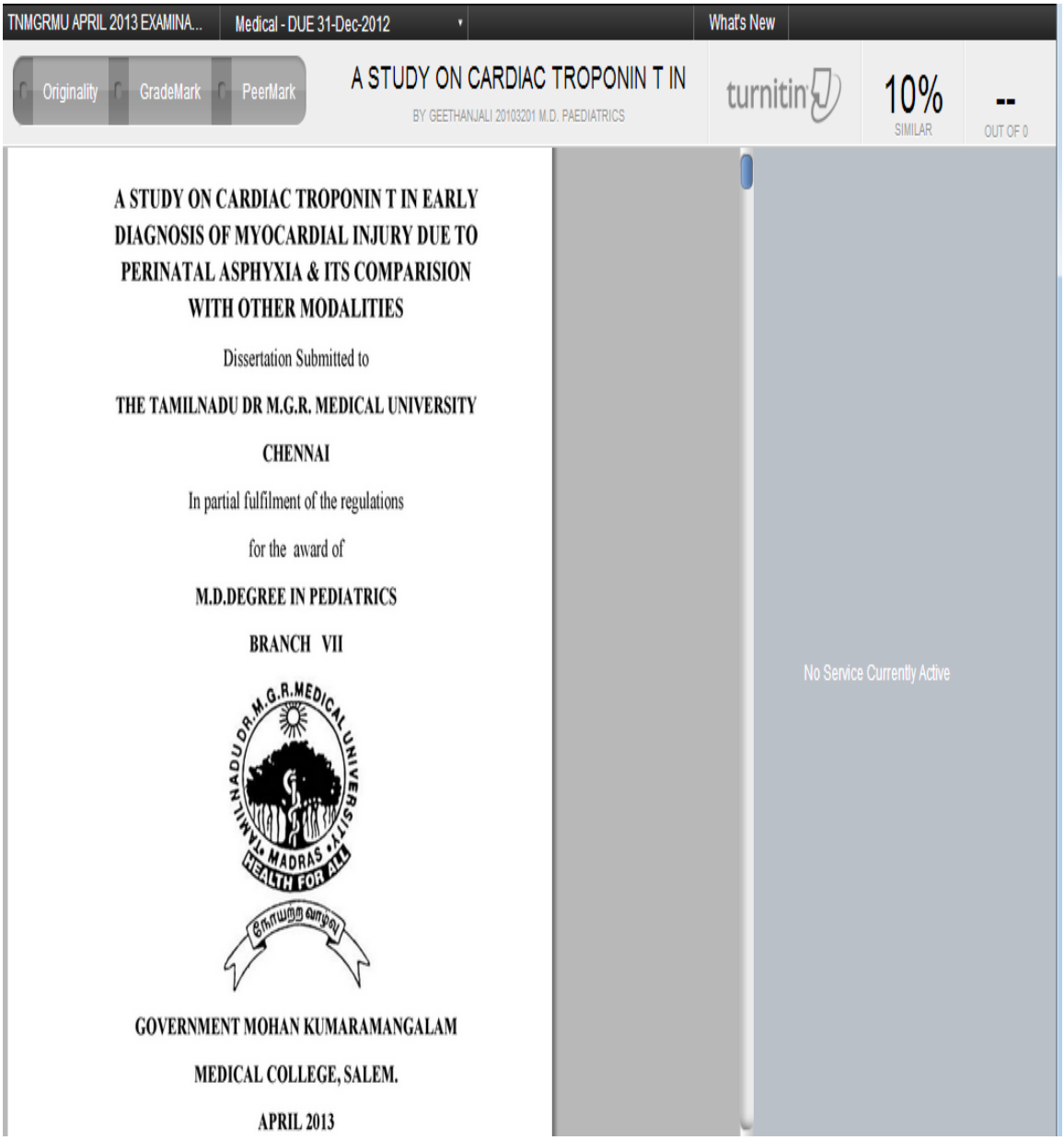

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ABBREVIATIONS USED IN THE STUDY

1.	**HIE**	Hypoxiac ischemic encephalopathy
2.	**ECG**	Electrocardiograph
3.	**ECHO**	Echocardiogram
4.	**cTnT**	Cardiac troponin-T
5.	**MOD**	Multi-organ dysfunction
6.	**CK-MB**	Creatine Kinase Myocardial bound
7.	**NNF**	National Neonatology Forum of India
8.	**PPHN**	Persistent pulmonary hypertension of newborn
9.	**EF**	Ejection fraction
10.	**DTI**	Doppler tissue imaging
11.	**TMI**	Transient myocardial ischemia
12.	**ANOVA**	Analysis of variance
13.	**PPV**	Positive predictive value
14.	**NPV**	Negative predictive value
15.	**NICU**	Neonatal intensive care unit
16.	**TR**	Tricuspid Regurgitation
17.	**LDH**	Lactate dehydrogenase

A STUDY ON CARDIAC TROPONIN T IN EARLY DIAGNOSIS OF MYOCARDIAL INJURY DUE TO PERINATAL ASPHYXIA & ITS COMPARISION WITH OTHER MODALITIES.

ABSTRACT

BACKGROUND:

Perinatal Asphyxia is a multi-system disorder and its effects are not limited to central Nervous System .Cardiac impairment occur in about 25% of neonates with asphyxia.Often cardiac impairment is overlooked due to the lack of sensitive diagnostic test.This study was done to evaluate the usefulness of Cardiac Troponin T card test as a reliable bedside test in diagnosing myocardial injury in perinatal asphyxia and its comparision with ECG and ECHO .

METHODOLOGY:

A Hospital based Prospective Analytical Study performed over 50 Asphyxiated Neonates admitted in our NICU from June 2012 to November 2012 .Myocardial dysfunction was evaluated using clinical, electrocardiography, echocardiography and Cardiac troponin-T card test.

RESULTS:

In our study, among the 50 neonates 32 had clinical evidence of myocardial injury. Significant association between HIE staging and all the diagnostic modalities ($p<0.005$). Mean ejection fraction between the

survivors and the non-survivors was statistically significant. Troponin Tcard test has the highest sensitivity of about 84.37 %, positive predictive value of 93.1% and negative predictive value of 76% in diagnosing myocardial injury in contrast to ECG and ECHO .In terms of specificity, troponin T has a better specificity (88.9%) compared to ECG but echocardiogram has higher specificity (94.4%)..Among all the diagnostic modalities used in this study,Troponin T best predicts the severity and outcome of Perinatal asphyxia.

CONCLUSION:

Troponin T card test is a valuable tool for early detection of myocardial injury due to perinatal asphyxia . In resource limited setting where the accessibility to 12 lead ECG, ECHO and aid of cardiologist are not available , Trop T card test will serve as an effective handy screening tool in diagnosing myocardial injury

KEYWORDS:

Perinatal Asphyxia, Cardiac Troponion T, Myocardial injury, ECG and ECHO.

INTRODUCTION

Perinatal asphyxia is a major root cause for neonatal mortality and long term morbidity next to Sepsis[1]. Incidence is about 1.0 -1.5% of live births comprising 20% neonatal death in India[1, 2]. Cardiac dysfunction can be figured out in 25% of neonates with asphyxia[1]. Documentation of cardiac abnormalities in birth asphyxia was done in early 1970s[3]. This study is done to evaluate the role of Troponin T card test as an early determinant of cardiac abnormalities in comparison to ECG and ECHO as the routine diagnostic tool. The consequence of perinatal hypoxia is not merely confined to central nervous system although impairment of other organ systems is worth considering. Cardiac abnormalities are nowadays under diagnosed and require high index of suspicion.

PERINATAL HYPOXIA

Asphyxia is a Greek terminology which implies loss of pulse. WHO describes perinatal asphyxia as “Failure to initiate or sustain respiration after birth”[4.] National Neonatology Forum of India has put forth definition of asphyxia as “when baby has gasping or inadequate breathing or no breathing at the end of one minute”[1].

NNF grades perinatal asphyxia as the second leading cause of Neonatal mortality. It records about 20 %[1].

Birth asphyxia refers to a condition during first and second stage of labour in which impaired gas exchange and blood supply leads to hypoxemia , hypercarbia and fetal acidosis[2]. Consequently CNS, kidney, heart and lung suffers hypoxic injury to about 28%, 50%, 25% and 23% respectively[1]. The magnitude of Multi- Organ Dysfunction Syndrome (MODS) ascertains early outcome of asphyxiated neonate. Long term sequelae are not linked to these organ dysfunctions with the exception of central nervous system, as it leads to hypoxic ischemic encephalopathy (HIE).

INCIDENCE

Perinatal asphyxia occurs in 1- 1.5 % of live births[2]. In India, neonatal death in each year due to perinatal asphyxia ranges from 2,50,000 to 3,50,000 generally within the first 3 days of life[1]. Intrauterine growth restricted babies, breech presentation post dated babies and infants of diabetic and toxaemic mothers, are at risk of perinatal asphyxia[2].

ETIOLOGY[2]

Events of asphyxia occur more during ante-partum and intra-partum period. Cardiac, respiratory and nervous system abnormalities lead to Asphyxia in the postpartum period as well.

RISK FACTORS FOR PERINATAL ASPHYXIA [2]

Deprivation of maternal oxygenation.

Diminished blood flow from mother to placenta,

Diminished blood flow from placenta to foetus.

Impairment of gas exchange across the placenta or at the foetal tissue level.

High fetal oxygen requirement.

ETIOLOGY OF HYPOXIC ISCHEMIA

MATERNAL FACTORS: Hypertension (acute or chronic), hypotension, infection (including chorioamnionitis), hypoxia from pulmonary or cardiac disorders, diabetes, maternal vascular disease, and in utero exposure to cocaine.

PLACENTAL FACTORS: Abnormal placentation, abruption, infarction, fibrosis.

Uterine rupture.

UMBILICAL CORD ACCIDENTS: Prolapsed, entanglement, true knot, compression.

Abnormalities of umbilical vessels.

FETAL FACTORS: Anemia, infection, cardiomyopathy, hydrops, severe cardiac/circulatory insufficiency.

NEONATAL FACTORS: CCHD, persistent pulmonary hypertension of the newborn (PPHN), cardiomyopathy, shock due to various reason.

APGAR SCORE[1,2]

Apgar score is applied for assessing the state of the neonate at 1 minute and then 5 minutes following birth. The neonate is surveyed for 5 key signs assigned a score of 0, 1 and 2.

	SIGN	0	1	2
1.	Heart rate	absent	Less than100 bpm	More than100 bpm
2.	Respiratory effort	absent	Slow	Good cry
3.	Muscle tone	limp	Some flexion of extremities	Active motion
4.	Reflex irritability	No response	Grimace	Cough or sneeze
5.	Colour	Blue, pale	Pink body, blue extremities	All pink

In accordance with NNF, moderate asphyxia is termed for babies having Apgar score 4 -6 and severely asphyxiated babies with Apgar score 0-3 at 1 minute.

MECONIUM ASPIRATION [2]

Acute or chronic hypoxia results in the passage of meconium in utero. In the presence of fetal stress, gasping by the fetus results in the aspiration of meconium before, during or immediately following delivery.

Meconium stained amniotic fluid occurs in 8-25 % of live births of which 5% develops meconium aspiration syndrome. Meconium aspiration can obstruct airways, interfere with gas exchange and cause respiratory distress. Severe meconium aspiration has been associated with increased risk of perinatal and neonatal mortality, severe acidemia and adverse neurological outcome.

PATHOPHYSIOLOGY[2]

Asphyxia causes a number of physiological and biochemical alteration. In mild asphyxia there is a transient increase in heart rate followed by decrease in heart rate, mild increase in blood pressure and central venous pressure in order to maintain the cerebral perfusion. There is a redistribution of cardiac output to brain, heart and adrenal glands (Diving Reflex).

With severe prolonged asphyxia, there is loss of pressure auto regulation and CO_2 vasoreactivity leading to cerebral hypo perfusion. It is further accentuated when there is cardiovascular abnormality with hypotension and decreased cardiac output. Decrease in cerebral blood flow leads to anaerobic metabolism and cellular energy failure due to increased glucose utilization in the Brain and fall in the concentration of

glycogen, phosphocreatine .Cellular dysfunction occurs as a result of diminished oxidative phosphorylation and ATP production.

MULTIORGAN DYSFUNCTION IN PERINATAL HYPOXIA[2]

ORGAN	EFFECT
Cardiovascular	Transient Myocardial ischemia, Tricuspid insufficiency, decreased left ventricular contractility, Pulmonary hypertension and systemic hypotension
Central nervous system	Hypoxic ischemic encephalopathy, seizures, cerebral enema, intracranial haemorrhage, hypotonia and spasticity
Pulmonary	Pulmonary hypertension, pulmonary haemorrhage, meconium aspiration and pulmonary edema
Kidneys	Acute tubular necrosis, cortical necrosis, renal failure, oliguria
Liver	Elevation of Hepato-cellular enzymes, altered metabolism, Hypoglycaemia and hyperbilirubinemia.
Gastro intestinal	Bowel ischemia and necrotizing enterocolitis
Hematologic	Disseminated intravascular coagulation, thrombocytopenia due to decreased production by the bone marrow

CARDIOVASCULAR EFFECTS OF BIRTH ASPHXIA[3]

Transient myocardial ischemia of the new born:

TMI is usually frequent in infants with perinatal asphyxia. It must be alleged in an asphyxiated newborn with respiratory distress or if the pulse is weak or absent pulses or baby with significant audible murmur. ECG shows flat or inverted T wave and ST depression. ECHO reveals decreased left ventricular contractility in particular the posterior wall. Prognosis can be determined by Left ventricular ejection fraction.

Transient tricuspid insufficiency in newborn:

Tricuspid insufficiency commonly contributes to cardiac murmur in asphyxiated newborn. Ischemic damage of tricuspid valve papillary muscle and pulmonary hypertension leads to Tricuspid insufficiency. Mostly tricuspid regurgitation tends to regress as underlying problems resolve.

Mitral Incompetence:

Mitral regurgitation is less frequent than tricuspid regurgitation. It is a vital pointer of myocardial ischemia. ECHO gives an evidence of impaired left ventricular contractility . It settles in due course in most instances.

Persistent pulmonary hypertension of newborn:

Due to high pulmonary vascular resistance there is right side to left side shunt in the fetal circulating pattern after birth. This shunt is between the path of ductus arteriosus and foramen ovale. PPHN presents usually with respiratory distress and cyanosis. Chronic fetal hypoxia leads to pulmonary smooth muscle hyperplasia consequently resulting in increased pulmonary vascular resistance. ECHO reveals dilated pulmonary artery and right heart with atrial and ventricular septae bulging into left atrium and ventricle respectively.

Dilated cardiomyopathy;

It comprises of cardiac dilatation, diminished cardiac contractility and congestive cardiac failure. Cardiac output is maintained by the ventricular dilatation and tachycardia ,despite diminished systolic shortening fraction.

Factors encompassing myocardial dysfunction :

Ischemia, hypoxia, pulmonary hypertension, lactic acidosis, Hypothermia, Hypocalcaemia , Hypercarbia, Anaemia and Polycythemia.

Congestive cardiac failure:

Transient myocardial ischemia leading to primary myocardial dysfunction causes congestive heart failure. The neonate with congestive cardiac failure presents with tachypnea tachycardia, hepatomegaly, diaphoresis, poor perfusion, feeding difficulties, growth failure, and cardiovascular collapse.

Cardiac dysarrthymia:

Ventricular fibrillation, tachycardia, sinus node arrest, extreme bradycardia is associated with severe asphyxia.

CARDIAC TROPONIN

Cardiac Troponin I and T are cardio regulatory proteins of the Tropomyosin complex that controls the calcium mediated interaction of actin and myosin. They are markers of myocardial injury[2]. Troponin T is not normally detectable in the serum and their levels are not influenced by sex, mode of delivery, gestation age and birth weight of the neonate[5,6]. Troponin T in maternal blood does not crosses the placenta owing to heavy molecular weight[7]. Cardiac Troponin T starts rising in the serum 2 -4 hours after myocardial injury, peaks at 48 hours and remains elevated for 7-10 days[8] .Furthermore they are competent prognostic indicator of mortality in asphyxiated newborn[5]. They are highly sensitive and specific in diagnosing myocardial injury in newborns with clinical and laboratory evidence of asphyxia[2]. Normal values of Troponin T in the newborn are 0 to 0.097µg/L [2]. Previously Serum Creatine Kinase Myocardial bound fraction was employed as a marker of myocardial injury in perinatal hypoxia. An elevation of CK-MB fraction >5% to 10 % might point towards myocardial injury[2]. Cardiac Troponin T has higher specificity and sensitivity in comparison to CK-MB.

ECG CHANGES

Electrocardiographic changes in perinatal asphyxia were categorized into four grades as put forth by **Jedikin et all**[9].

Grade I – Flattening or inverted T waves in one or two leads excluding aVR.

Grade II - Flattening or inverted T waves in three or more leads excluding aVR.

Grade III- Grade II with more than 2mm of ST segment elevation or depression in minimum of two chest leads or abnormality of q wave described as more than 0.02secs elevation or more than 25% amplitude of r wave in one anterior or three related chest leads

Grade IV- abnormal q waves with classical segmental infarction or elevated ST segments or complete LBBB.

ECHOCADIOGRAPHIC CHANGES

Echocardiographic changes witnessed in asphyxiated newborn with myocardial injury comprises of valvular regurgitation tricuspid / mitral valve incompetence, Right ventricular hypokinesia and Left ventricular hypokinesia supported by low ejection fraction(EF), pulmonary hypertension and Right Atrial/Right ventricle dilatation[10]. Ejection

fraction is related to the change in volume of the Left ventricle with cardiac contraction. Normal mean ejection fraction is 66% with a range of 56% to 78 %.[11]

CLINICAL EVIDENCE OF MYOCARDIAL INJURY[1,2]

In perinatal asphyxia there is loss of cerebral auto regulation and it is pressure passive. Myocardial injury is manifested as hypotension and decreased cardiac output which further compromises the cerebral perfusion. Adequate end organ perfusion is measured by Capillary filling time < 3 seconds(CFT), Systemic mean arterial Blood pressure to the bare minimum of 45 to 50mm Hg, urine output >1ml/kg/hour and Normal Central venous pressure 5-8 mm Hg in term neonates(CVP). Decreased CVP and prolonged CFT denote reduced intravascular volume and need for inotropic support.

REVIEW OF LITERATURE

1. Shah et al [12]

Multi-organ dysfunction (MOD) in and due to perinatal asphyxia was studied by Shah et al. Out of 132 infants who had perinatal asphyxia, 80 infants had worse outcome (either death or neuro-developmental disability) and only 50 had good outcome. Almost all infants had at least one organ involvement along with HIE. 64-86% of infants with worse outcome had multi-organ dysfunction (renal, cardio-vascular, pulmonary and hepatic dysfunction) whereas 58-88% of the infants had multi-organ dysfunction. Multi-organ dysfunction was present in all infants with severe perinatal asphyxia. However, there was no association between outcome of the infants and presence of multi-organ dysfunction. Furthermore, there was no relationship between outcome and individual or any combination of organ involvement.

2. Kanik et al1[3]

The importance of myocardial dysfunction due to perinatal asphyxia causing hypoxic-ischemic encephalopathy (HIE) was studied by kanik et al. They also studied the myocardial involvement as a predictor of mortality in neonates with hypoxic-ischemic encephalopathy. 34 full term neonates were classified and staged according to Sarnat and sarnat

classification. Electrocardiogram (ECG), Echocardiogram (Echo) was done in the 24-48 hours after birth, while serum Troponin-I and CK-MB were measured at the time of delivery and day 3 of postnatal life. Among the 34 cases of HIE, 19 were in stage 1, while 9 and 6 were in stage 2 and 3 respectively. 9 neonates succumbed to the disease. Although 13 neonates had ECG changes related to perinatal asphyxia only one child had ECHO changes. Levels of Troponin-I were higher among cases who succumbed than who survived but CK-MB did not have any predictive value. This study highlighted the significance of evaluating cardiac involvement in newborns with HIE.

There are various studies done to detect myocardial damage in infants born with perinatal birth asphyxia. Of the diagnostic modalities studied to detect myocardial involvement in asphyxiated neonates, the most common are electrocardiography, echocardiography and Troponin-T. Elevation of cardiac Troponin-T which is the structural protein that binds the Tropomyosin molecular strand to the Troponin complex which is widely used as a specific biochemical marker for diagnosis of myocardial infarction in adults.

3. Costa et al1[4]

Costa et al studied the association between echocardiography findings and cardiac Troponin T (cTnT) concentrations in newborn

infants with perinatal asphyxia. 29 infants with asphyxia were compared with 30 infants in the control group in terms of ECG, echocardiogram and cardiac Troponin T (cTnT) concentrations. Among various echocardiographic parameters, left ventricular output (LVO) and stroke volume (stroke volume) were significantly lower in the asphyxiated infants and cardiac Troponin T (cTnT) concentrations were significantly higher when compared to control groups: 0.15(0.10 -0.23) vs. 0.05 (0.02 - 0.13), p <0.001. Infants with myocardial damage due to asphyxia had significantly higher cTnT when compared to control group (0.20 (0.11-0.28) vs. 0.11 (0.05-0.14 uGu/L)) and so they concluded that cardiac Troponin is a valuable tool in evaluating myocardial injury in newborns with birth asphyxia.

4. Rajakumar et al[15]

Rajakumar et al studied the elevation of cardiac enzymes in detecting myocardial damage in 30 term neonates with perinatal asphyxia (cases) in comparison with 30 term neonates without asphyxia (controls). Out of 30 cases, 23 had myocardial injury when compared to 1 baby in the control group. The mean serum level of Troponin-T were 0.22±0.28 in the cases and 0.003±0.018 in the control while mean serum level of CK MB were 121 ± 77.4 IU/L in the cases and 28.8 ± 20.2 IU/L in the control. It was inferred that Cardiac Troponin-T had better sensitivity

and specificity than CK-MB in evaluating myocardial dysfunction also the level of Troponin-T correlated with severity and outcome of the case.

5. Kilic et al[16]

Kilic et al showed that asphyxiated neonates have higher TnT levels in cord blood and venous blood samples than controls ($p<0.001$). The controls were 30 healthy neonates between 34-40 gestational ages and the study group consisted of 30 neonates affected with perinatal asphyxia between 32-40 weeks of gestation. Besides cTnT, CK-MB levels in venous and cord blood were also elevated in the study group and were higher than control group ($p< 0.01$). Among the cases who succumbed, 11 neonates (36%) had high TnT levels, low umbilical arterial pH ($p<0.05$) than those between the survivors and the deceased. According to Kilic et al, cardiac Troponin-T had 100% specificity for detecting myocardial damage due to asphyxia in neonates in comparison with 96% specificity of CK-MB.

6. Guenes et al[17]

Guenes et al showed that infants who had severe asphyxia also had high cardiac Troponin-T when compared with grade 1, 2 asphyxiated and normal neonates in first 4 hours of life (0.34 ± 0.21 ng/ml vs. 0.07 ± 0.003 ng/ml, 0.12 ± 0.07 ng/ml, 0.04 ± 0.02 ng/ml, respectively). In

infants with severe asphyxia Troponin-T remained high on day 3 and 7. CK-MB levels were significantly higher in infants with grade 2, 3 asphyxia than in infants with grade 1 asphyxia and healthy neonates in first 4 hours of life but it was not significantly higher on day 3. Echocardiographic pathology was detected in 12 infants with grade 3 asphyxia on B mode echo image on day 1 but no echo findings were present in the day 7 and day 15 in any of the groups. They concluded that reversible cardiac changes were significantly present in infants with severe asphyxia and Troponin T can be used as a good tool to detect degree of cardiac injury in the first week of life

7. Agrawal et al[18]

A hospital based prospective study done by Agrawal et all showed that early detection of HIE using abnormal ECG and cardiac enzymes can help in better management and survival of the neonate. They studied 60 term neonates who had birth asphyxia and used clinical, ECG, CK total, CK-MB and Troponin-I for assessment of myocardial dysfunction. Among the 60 infants, 13 had mild HIE whereas 27 and 20 had moderate and severe HIE, respectively. ECG findings pertaining to perinatal asphyxia were found in 46 cases. Elevated levels of CK-total and CK-MB were present in 54 and 52 infants respectively while Troponin-I was elevated in 48 cases. ECG findings and elevations in cardiac enzymes

were significantly associated with the different grades of HIE (p= 0.002, 0.02, <0.001, 0.004, respectively). The non-survivors when compared to survivors had significantly higher levels of CK-MB (p=0.018) and Troponin-I (p=0.008) and also high proportion of them had abnormal ECG changes pertaining to perinatal birth asphyxia.

8. Matter et al[19]

Matter et al studied 25 asphyxiated term and 20 term non-asphyxiated neonates using Doppler echocardiography and Doppler tissue imaging (DTI) during first 72 hours of life and also studied the correlation between DTI and serum Troponin-T concentration. Asphyxiated neonates had significantly high right and left ventricular (RV and LV) Tei indexes (RV mean ± SD: 0.45 ± 0.05 vs. 0.28 ± 0.05, P < 0.001 and LV mean ± SD: 0.51 ± 0.04 vs. 0.38 ± 0.04, P < 0.001, respectively) besides having significantly lower mitral and tricuspid systolic (Sm) velocities (mean ± SD: 5.06 ± 0.89 vs. 6.89 ± 0.94 cm/s, P < 0.001and5.78 ± 0.58vs. 6.69 ± 0.87 cm/s, P < 0.001 respectively). Asphyxiated neonates had significantly high cTnT concentrations when compared to non-asphyxiated neonates with median range: 0.17 (0.05–0.23) vs. 0.03 (0–0.07) μg/l, P < 0.001. High cTnT concentrations were positively correlated with LV Tei index (r = 0.67, P < 0.001) and RV Tei index (r = 0.68, P < 0.001) and

negatively with the mitral systolic (Sm) velocity ($r = –0.68$, $P < 0.001$). High cTnT concentrations were negatively correlated with tricuspid systolic (Sm) velocity ($r = –0.41$,$P = 0.01$). Although DTI measurements and fractional shortening (FS) did not have any predictive value, elevated serum cTnT concentrations predicted mortality significantly in asphyxiated infants. When compared to conventional echocardiography, the DTI technique had high sensitivity in detection of myocardial damage due to perinatal asphyxia.

9. Turker et al[20]

Turker et al studied the usefulness of cord blood troponin I as a predictor of short term outcome in perinatal asphyxia. 54 newborns born consecutively with HIE were included in the study group while the control group consisted of 50 consecutive infants without asphyxia. Arterial blood gas (ABG) analysis was done on venous and arterial cord blood samples and venous blood levels of cardiac Troponin-I and creatine kinase and CK-MB were analysed on day 3 and day 7 of life. The study infants had significantly high cord blood cTnI levels than the infants in the control group ($p < 0.001$). Cardiac Troponin-I levels were significantly high in non-survivors than in infants who survived (5.9 ng/ml vs 1.6 ng/ml respectively; $p < 0.001$). They concluded that cTnI

levels can be used as a sensitive tool for predicting severity of HIE and death in infants with perinatal asphyxia.

10. Trevisanuto D et al[21]

Trevisanuto D et al also studied importance of Troponin-I as a marker of myocardial ischemia due to perinatal asphyxia. With a control group of 39 infants, 13 infants were studied who had asphyxia using blood samples for assessing umbilical pH, creatinine and serum alanine and aspartate aminotransferase levels. They found that study group infants had higher levels of cTnI when compared to control group (0.36 ug/ml (0.05-11) vs. 0.04 ug/ml (0.04-0.06); $p < 0.01$). However there was no correlation between elevation in cTnI levels and other markers of asphyxia.

11. Boo et al[22]

A comparative study done by Boo et al showed that cord blood serum concentration of cardiac troponin-T was significantly higher in the infants with severe birth asphyxia and non-survivors. The study group consisted 50 term neonates with clinical features of asphyxia and serial measurements of cTnT and CK-MB concentrations measured at birth and also at 12, 24, and 48 hours of life after birth using chemiluminescence immune-assay. The control group consisted 50 term neonates without

clinical features of asphyxia and all these infants were followed up till their discharge from hospital or death. Infants in the study group had significantly higher cord blood cTnT and CK-MB at birth ($p < 0.0001$) and among them, neonates with low ejection fraction of < 60% also had high enzyme levels ($p < 0.05$). In addition, asphyxiated infants with congestive cardiac failure during 48 hours of life and infants who succumbed within 48 hours of life had significantly high concentration of cardiac troponin-T ($p < 0.04$ and $p < 0.0001$, respectively) but not CK-MB.

12. Clark et al[23]

While these studies indicated the importance of cardiac troponin-T (cTnT) in evaluating myocardial damage in asphyxiated infants, Clark et al investigated its role in critically ill neonate admitted to intensive care unit (PICU) without congenital heart disease. They had 107 consecutive infants, out of which 47 were in PICU and 60 healthy controls. The median (IQR) of cTnT levels in PICU and control group infants were 18 (10-60 pg/ml) and 10 (10-10pg/ml) ($p < 0.001$) respectively. They found out a positive correlation between cTnT levels and the paediatric index of mortality score ($r=0.41$, $p=0.004$). However, the correlation lost its significance when applied for age. Age was a important factor as infants

under 1 month had higher cardiac troponin-T (cTnT) level than older patients (p= 0.013).

They concluded that, when compared to the older infants in the control group, the infants in PICU have higher levels of cardiac troponin –T in their neonatal period despite of not having more severe disease.

AIM OF THE STUDY

1. To evaluate CARDIAC TROPONIN T card test as a reliable bedside test in diagnosing myocardial injury in perinatal asphyxia.
2. To compare the sensitivity of ECG and ECHO with Troponin T in diagnosing myocardial injury.
3. To determine the severity of myocardial damage and outcome of perinatal hypoxia.

MATERIALS AND METHODS

Study place : Neonatal ward, Govt. Mohan kumaramangalam Medical College & Hospital, Salem 1.

Study Duration : 01.06.2012 to 30.11.2012

Study Population : Asphyxiated Newborn admitted in our NICU.

Study Design : Hospital based Prospective Analytical Study

Sample Size : 50 asphyxiated infants.

INCLUSION CRITERIA

1.Term Babies

2.Apgar Score ≤ 6 at 1 minute

EXCLUSION CRITERIA

1. Preterm Babies

2. Babies with congenital Anomalies

3. Mild Asphyxia (Apgar > 7)

4. Babies who died before evaluation.

STUDY PROTOCOL

Ethical committee clearance was obtained to conduct the study in our Hospital. Informed consent was obtained from the parents and caregivers before including the neonates in the study. There is no added risk or harm to the baby because of the study.

A detail history was elicited for all recruited babies and was thoroughly examined.HIE staging of asphyxiated newborn were done using Sarnat and Sarnat classification. Details of the gestational age, mode of resuscitation, Apgar score, birth weight and maternal complications were documented. Clinical progression of the neonates was closely observed.

12 lead Electrocardiograph was recorded on 2nd day of life and it was100% magnified and photocopied for the study.

Two dimensional, M mode, Doppler Echocardiogram was performed using Philips 2011 Doppler Echocardiogram machine on 2nd day of life to identify the cardiac abnormalities in perinatal hypoxia.

Cardiac Troponin T (cTnT) card test was done using ROCHE Cardiac, COBAS KIT. This test is intended for the qualitative determination of Cardiac Troponin t in anti-coagulated venous blood. The test was done on Day 1 of life between 12-24 hrs. The Troponin T

sensitive test is designed to yield a positive result for cardiac Troponin concentrations ≥ 0.08ng/ml. The presence of two lines in the read window i.e. test line and control line indicates the presence of cardiac Troponin T ≥ 0.08ng/ml. Presence of control line alone indicates cTnT was <0.08ng/ml. Even a very faint line indicates a positive test.

The cases were followed up. After 6 weeks of follow up, ECG and ECHO were performed if it was abnormal earlier.

CASE DEFINITION OF MYOCARDIAL INJURY

The paradigm for cardiovascular impairment in perinatal hypoxia as proposed by Shah et al[12] was systemic hypotension requiring vasopressors (dopamine, dobutamine) to sustain mean arterial pressure of 45 -55mm Hg for more than 24 hours.

All the diagnostic modalities in the study like ECG, Echocardiogram and troponin-T card test were evaluated for sensitivity and specificity in comparison with clinical diagnosis of myocardial injury.

STATISTICAL ANALYSIS

All the information and test results from the cases were collected and recorded in a master chart. Data analysis was done using **statistical package for social sciences (SPSS) software**, version 20.

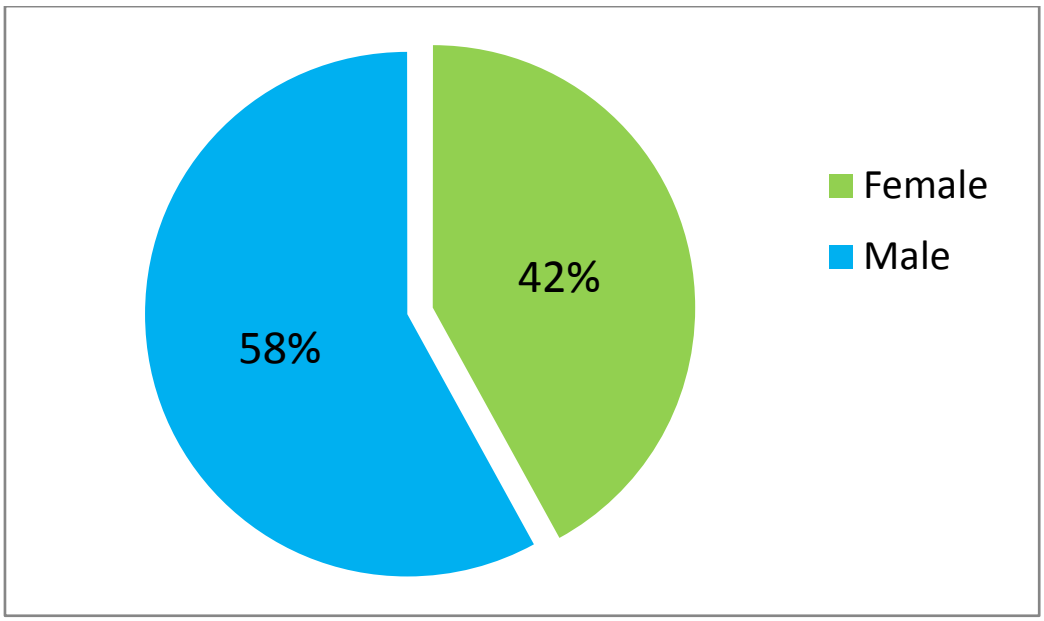
Almost all the study variables were categorical except for ejection fraction which was a continuous variable and it was normally distributed.

Data was analyzed using appropriate statistical methods and represented by various tables, graphs, diagrams etc.

Various statistical tests of significance were applied according to the type of variable and all the tests of hypothesis were done using the software. For all the tests of significance, ‘p’ value was considered significant if it was less than 0.05.

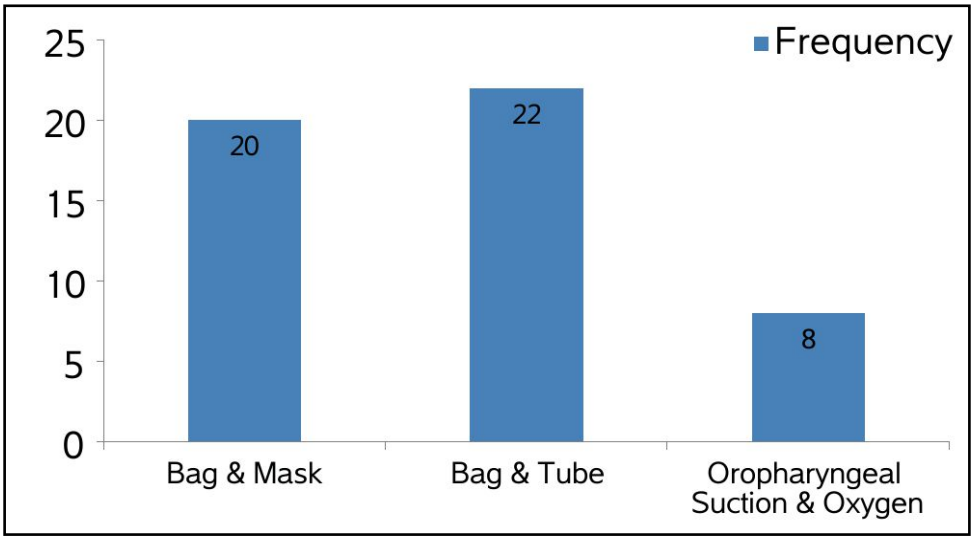
RESULTS

Fig 1 : Gender distribution



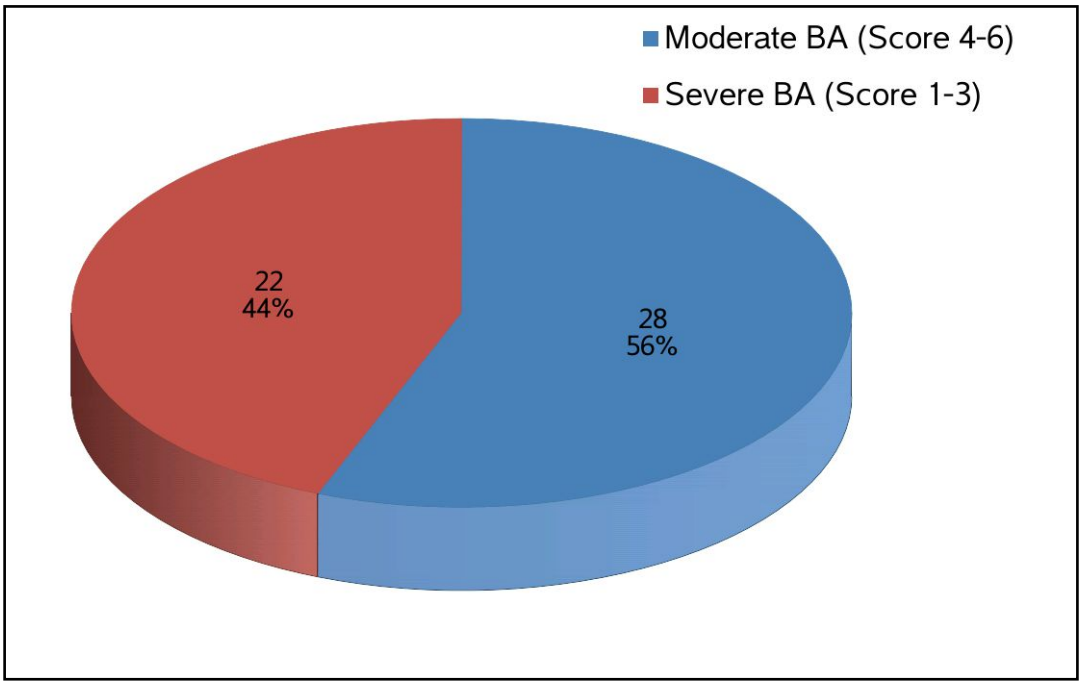
Among the 50 new-borns in the study, 29 (58%) were male and 21(42%) were female neonates.

Fig 2 : Mode of resuscitation



In the study population, 44% of neonates were resuscitated using bag & tube and 40% bag & mask ventilation.

Fig 3: Apgar score at 1 minute.



In the study population, 56% had moderate birth asphyxia, while 44% had severe birth asphyxia.

Table 1: Distribution of new-borns according to Hypoxic-Ischemic Encephalopathy (HIE) Staging.

HIE staging	Frequency	Percentage (%)
1	6	12
2	25	50
3	19	38
Total	50	100

About 50% of the infants were in stage-2 and 38% were in stage -3 of HIE staging, according to Sarnat and Sarnat classification.

Table 2 : Distribution of the study population according to ECG changes observed

ECG	*N*	*%*
No changes	**25**	**50 %**
flat or inverted t waves in 1 or 2 leads (I)	**17**	**34%**
flat or inverted t waves in >=3 leads (II)	**8**	**16%**
II+ST wave changes (III)	**0**	**0%**
Abnormal Q waves (IV)	**0**	**0%**
Total	**50**	**100%**

Among the study population,

- 50% of the infants had no ECG changes.
- 34% had flat or inverted T waves in 1 or 2 leads
- 16% had flat or inverted T waves in 3 or more leads.

Table 3: Distribution of the study population according to Echocardiographic abnormalities

ECHO	*N*	*%*
Mitral regurgitation	0	0 %
Normal	29	58%
Pulmonary hypertension	1	2 %
PHT And Tricuspid regurgitation	2	4%
Tricuspid Regurgitation	18	36%
Total	50	100%

- About 58% of the infants had normal ECHO findings.
- Among Echocardiographic abnormalities, Tricuspid Regurgitation (36%) was the commonest finding observed in the study.

Table 4: Distribution of study population according to Left ventricular Ejection fraction and HIE staging.

HIE staging	N	Mean	Std. Deviation	95% Confidence Interval for Mean	Range
Stage 1	6	65.83	3.251	62.42 - 69.24	61 – 71
Stage 2	25	61.60	9.574	57.65 - 65.55	35 – 70
Stage 3	19	52.95	10.368	47.95 - 57.94	34 – 70
Total	50	58.82	10.431	55.86 - 61.78	34 - 71

One-way ANOVA (Analysis of variance) was done to compare means between 3 groups in HIE staging and equal variance was assumed according to Levene's test.

Anova test

F statistic – 6.417

P value (significant at 0.05 level): 0.003

The difference between means of 3 groups was statistically significant.

Bonferroni post hoc test

Multiple comparisons were made using post hoc test.

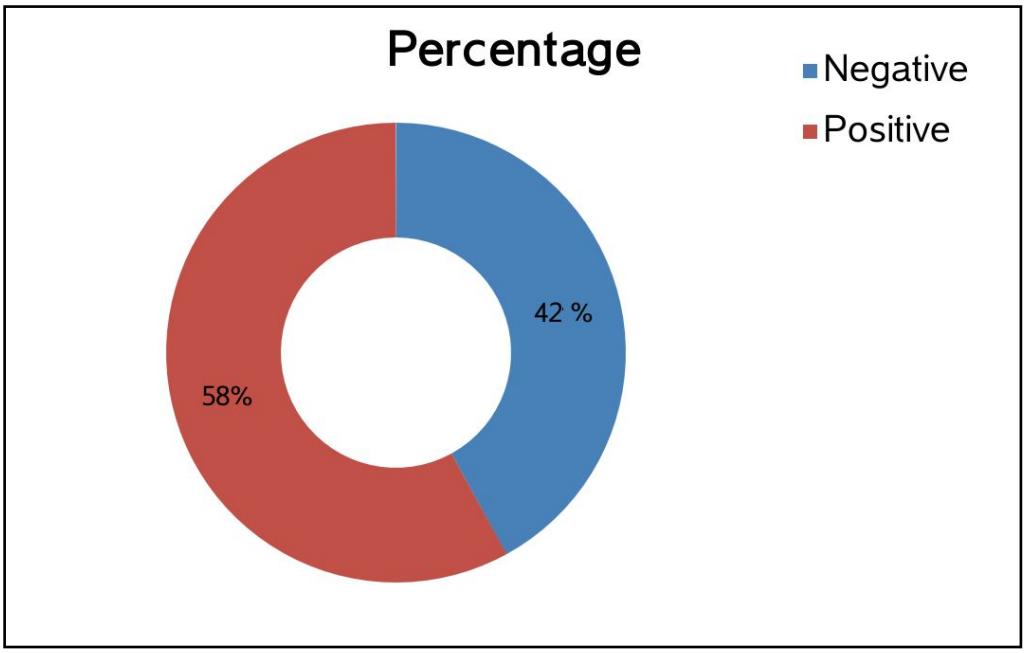
TEST	Mean Difference	P value
stage 1 Vs stage 2	4.233	0.987
stage 1 Vs stage 3	12.886*	0.016
stage 2 Vs stage 3	8.653*	0.013

- The difference between means was not significant between stage 1 and stage 2 (p = 0.98)
- The difference between means was significant between stage 1 and stage 3 (p = 0.016)
- The difference between means was significant between stage 2 and stage 3 (p = 0.013)

Table 5: Troponin T card test results

Troponin T card test	Frequency	Percentage (%)
Negative	21	42%
Positive	29	58%
Total	50	100%

Fig 4 : Showing percentage of infants according to Troponin-T card test



In the study group, 54% of neonates had elevation in troponin T levels as evidenced by a positive card test.

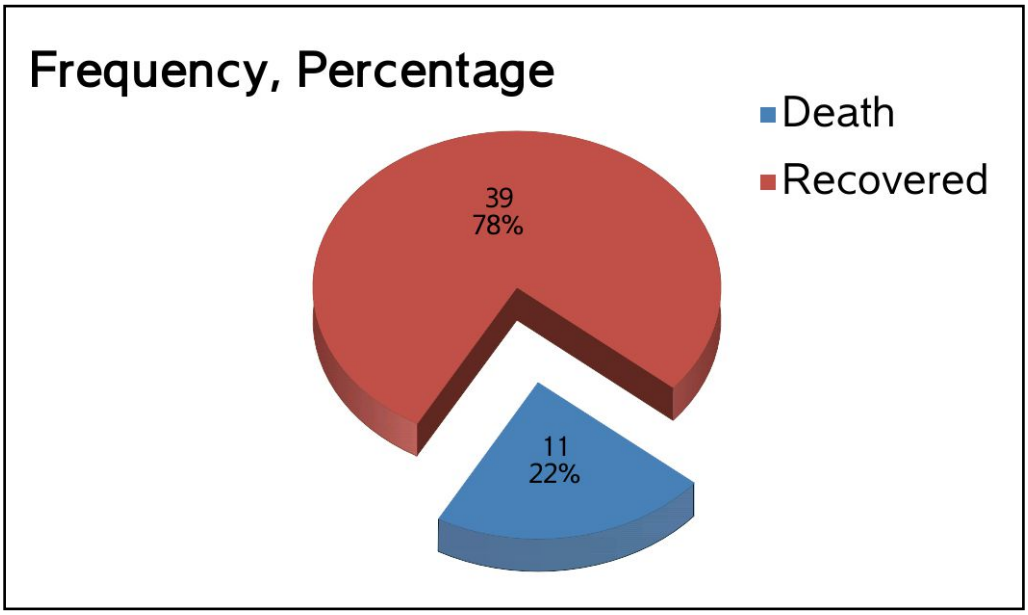
Table 6: Clinical diagnosis of myocardial injury

Myocardial injury	Frequency	Percentage (%)
Present	32	64
Absent	18	36
Total	50	100

Infants who had signs of circulatory shock i.e. systemic hypotension and those who needed inotropic support for more than 24 hours, were diagnosed to have myocardial injury clinically.

About 32 infants were diagnosed with myocardial injury.

Fig 5: Pie distribution of Final outcome



Among the 50 new-borns in the study, 11 babies expired and the mortality rate was 22%.

Table 7: Correlation between HIE staging and ECG changes

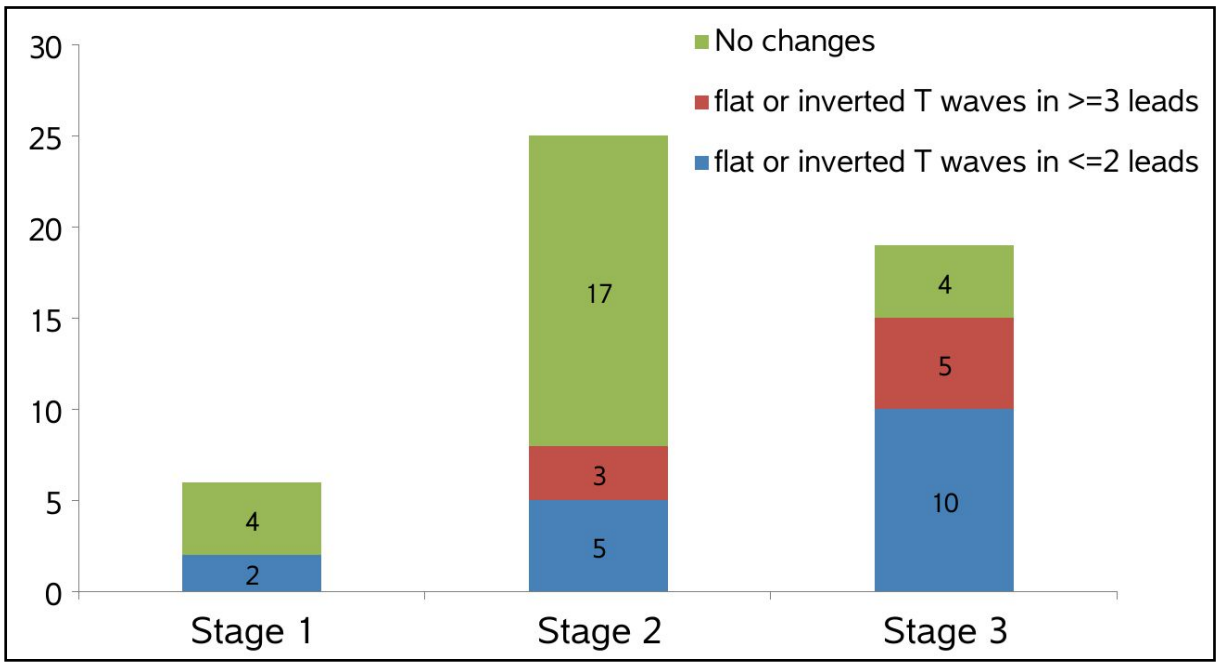
HIE STAGING	ECG n(%)			Total n(%)
	flat or inverted T waves in >=3 leads	flat or inverted T waves in 1 or 2 leads	No changes	
1	0 (0%)	2 (33.3%)	4 (66.7%)	6 (100%)
2	3 (12%)	5 (20%)	17 (68%)	25 (100%)
3	5 (26.3%)	10 (52.6%)	4 (21.1%)	19 (100%)
Total	8 (16%)	17 (34%)	25 (50%)	50 (100%)

Pearson Chi-Square value: 10.993

P value (significant at 0.05 level) : 0.027

There is a significant association between HIE staging and presence of ECG changes in the study population.

Fig 6 : Bar depiction of correlation between HIE staging and ECG changes



HIE Staging

Table 8: Correlation between HIE staging and Echocardiographic changes.

HIE STAGING	Echocardiogram n (%)		Total n (%)
	Abnormal	Normal	
1	0 (0%)	6 (100%)	6 (100%)
2	8 (32%)	17 (68%)	25 (100%)
3	13 (68.4%)	6 (31.6%)	19 (100%)
Total	21 (42%)	29 (58%)	50 (100%)

Pearson Chi-Square value: 10.816

P value (significant at 0.05 level): 0.004

There was a significant association between HIE staging and presence of ECHO changes in the study population.

Fig 7: Bar depiction of Echocardiographic abnormalities in each HIE Stages.

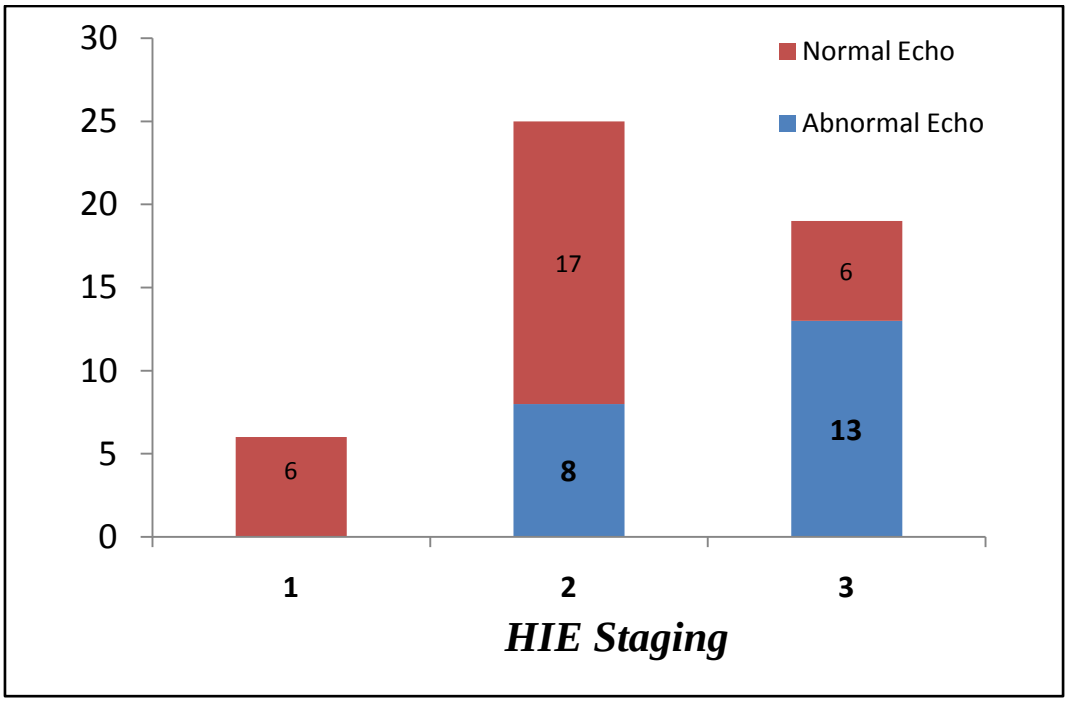


Table 9: Correlation between HIE staging and Troponin-T card test.

HIE STAGING	Troponin T card test n (%)		Total n (%)
	Positive	Negative	
1	1 (16.7%)	5 (83.3%)	6 (100%)
2	10 (40%)	15 (60%)	25 (100%)
3	18 (94.7%)	1 (5.3%)	19 (100%)
Total	29 (58%)	21 (42%)	50 (100%)

Pearson Chi-Square value: 18.060

P value (significant at 0.05 level) : <0.001

There was a significant association between HIE staging and positive Troponin T card test in the sample population.

Fig 8 : Bar depiction of Troponin card test in each HIE stage.

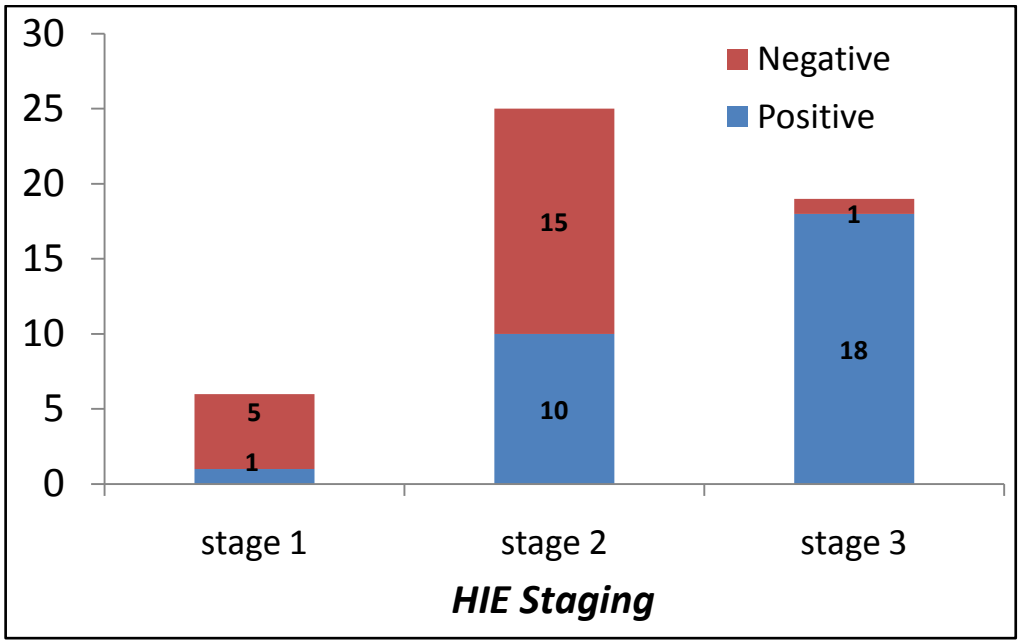


Table 10: Correlation between HIE staging and Clinical diagnosis of myocardial injury

HIE STAGING	Myocardial injury n(%)		Total n(%)
	Present	Absent	
1	2 (33.3%)	4 (66.7%)	6 (100%)
2	13 (52%)	12 (48%)	25 (100%)
3	17 (89.5%)	2 (10.5%)	19 (100%)
Total	32 (64%)	18 (36%)	50 (100%)

Pearson Chi-Square value: 9.363

P value (significant at 0.05 level): 0.009

There was a significant association between HIE staging and presence of myocardial injury clinically in the study population.

Fig 9: Bar depiction of myocardial injury in all HIE stages

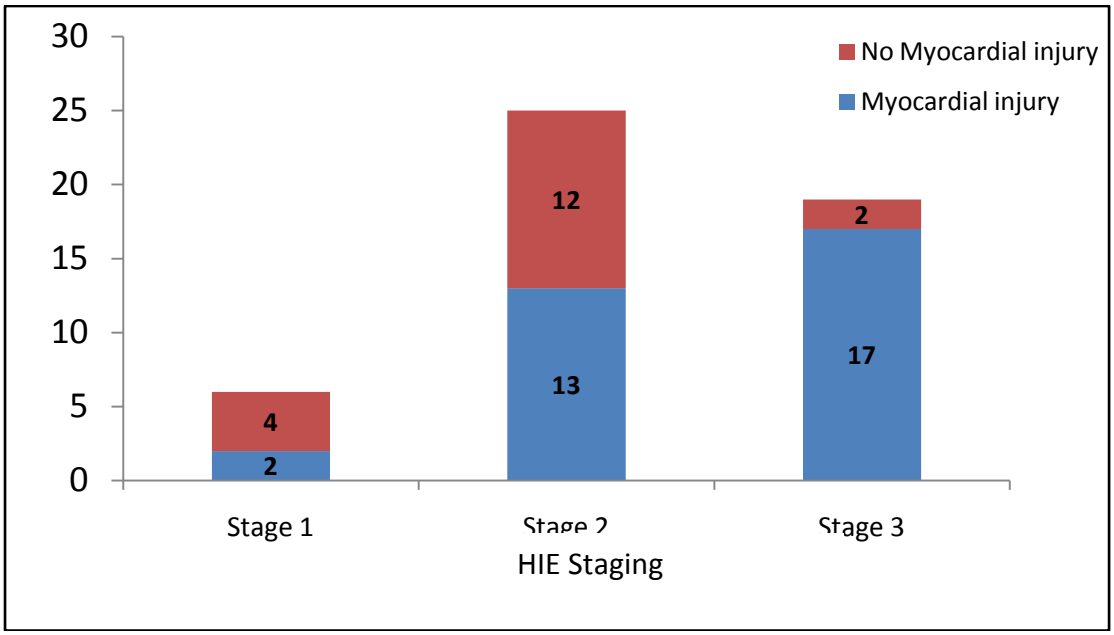


Table 11: Correlation between HIE staging and mortality.

HIE STAGING	OUTCOME n (%)		Total n (%)
	Recovered	Death	
1	6 (100%)	0 (0%)	6 (100%)
2	22 (88%)	3 (12%)	25 (100%)
3	11 (57.9%)	8 (42.1%)	19 (100%)
Total	39 (78%)	11 (22%)	50 (100%)

Pearson Chi-Square value : 7.625

P value (significant at 0.05 level) : 0.022

There was a significant association between HIE staging and mortality in the study population.

Table 12: Correlation between Ejection fraction and Mortality.

OUTCOME	N	Mean	Std. Deviation
Recovered	39	62.15	8.031
Death	11	47.00	9.539
Total	50	58.82	10.431

- **Student t test** was done to compare means between 2 outcome groups.
- Mean difference - 15.15
- ‘ t ‘ statistic – 5.305
- P value (significant at 0.05 level) - <0.001
- 95% confidence interval of the difference @ 9.41 – 20.89

The difference between the mean ejection fraction between the survivors and the non-survivors was statistically significant.

Table 13: Summary of results obtained in various diagnostic modalities used in the study and their correlation with outcome.

Diagnostic modality		OUTCOME		
		Recovery	Death	Total
ECG	flat or inverted t waves in >=3 leads	4	4	8
	flat or inverted t waves in 1or2 leads	12	5	17
	No changes	23	2	25
ECHO	Normal	26	3	29
	Pulmonary HT & Tricuspid regurgitation	0	2	2
	Pulmonary hypertension	1	0	1
	Tricuspid regurgitation	12	6	18
Troponin T card test	Positive	18	11	29
	Negative	21	0	21
Clinical diagnosis	Myocardial Injury	21	11	32
	No Myocardial Injury	18	0	18

Table 14: Association (Chi-square test) between outcome and various diagnostic modalities

Variables	*Pearson Chi-square*	**p value*
ECG vs Outcome	5.711	0.017
Echo vs Outcome	5.466	0.019
Troponin-T vs Outcome	10.212	0.001
Clinical diagnosis vs Outcome	7.933	0.005
Ejection fraction vs Outcome	5.305**	<0.001**

p value significant if < o.o5.

****t statistic and p value from student "t" test.***

All the diagnostic modalities used for diagnosing myocardial damage in the asphyxiated infants were significantly associated with outcome as evidenced by the chi-square test. This indicates that these tests can be used as one of the predictors of mortality due to myocardial involvement in infants with perinatal asphyxia.

Table 15. Comparison of Troponin-T with clinical diagnosis of myocardial damage

Troponin-T test	**Clinical diagnosis** n (%)		**Total** n (%)
	Myocardial injury	No myocardial injury	
Positive	27 (93.1%)	2 (6.9%)	29 (100%)
Negative	5 (23.8%)	16 (76.2%)	21 (100%)
Total	32 (64%)	18 (36%)	50 (100%)

Pearson Chi-square – 25.384 ($p < 0.001$)

Odds ratio – 43.20

95% confidence interval @ 7.48 - 249.23.

Sensitivity = 27/32 = 84.37

Specificity = 16/18 = 88.88

Positive predictive value = 27/29 = 93.10

(Probability of clinical disease when troponin-t test is positive)

Negative predictive value = 16/21 = 76.19

Infants who had positive troponin-T test had 43 times more risk of having myocardial injury clinically when compared to infants with negative troponin-T test

Table 16. Comparison of ECG abnormalities with clinical diagnosis

ECG	**Clinical diagnosis** n (%)		**Total** n (%)
	Myocardial injury	No myocardial injury	
ECG changes	20 (80%)	5 (20%)	25 (100%)
No ECG changes	12 (48%)	13 (52%)	25 (100%)
Total	32 (64%)	18 (36%)	50 (100%)

Pearson Chi-square – 5.556 (p = 0.018)

Odds ratio – 4.333

95% confidence interval @ 1.235 -15.206

Sensitivity = 20/32 = 62.50 %

Specificity = 13/18 = 72.22 %

Positive predictive value = 20/25 = 80 %

Negative predictive value = 13/25 = 52 %

Infants who had ECG changes have 4 times more risk of having myocardial injury clinically when compared to infants with no ECG changes.

Table 17. Comparison of Echocardiogram abnormalities with clinical diagnosis

ECHO	**Clinical diagnosis** n (%)		**Total** n (%)
	Myocardial injury	No myocardial injury	
Abnormal	20 (95.2%)	1 (4.8%)	21 (100%)
Normal	12 (41.4%)	17 (58.6%)	29 (100%)
Total	32 (64%)	18 (36%)	50 (100%)

Pearson Chi-square – 15.335 ($p < 0.001$)

Odds ratio – 28.33

95% confidence interval @ 3.334 - 240.81.

Sensitivity = 20/32 = 62.5 %

Specificity = 17/18 = 94.4 %

Positive predictive value = 20/21 = 95.23 %

Negative predictive value = 17/29 = 58.62 %

Infants who had echocardiographic changes pertaining to perinatal asphyxia have 28 times more risk of having myocardial injury clinically when compared to infants with normal echocardiographic changes

Table 18. Comparison of ECG, Echocardiogram and troponin-T test with clinical diagnosis

Test	Sensitivity	Specificity	Positive predictive value (PPV)	Negative predictive value (NPV)
ECG	62.5 %	72.2 %	80 %	52 %
Echo	62.5 %	94.4 %	95.2 %	58.6 %
Troponin-T	84.4 %	88.9 %	93.1 %	76.2 %

The above table depicts the parameters for each test, calculated using clinical diagnosis as comparison, for detection of myocardial damage in the asphyxiated infants.

In terms of sensitivity, troponin T has a better sensitivity (84.4%) and when compared to ECG and echocardiogram.

In terms of specificity, troponin T has a better specificity (88.9%) when compared to ECG but echocardiogram has higher specificity (94.4%).

Probability of progression to clinical signs or shock due to myocardial injury in a infant with positive troponin-t test (PPV) is very high (93.1%).

Furthermore, probability of not progressing to clinical signs or shock following a negative troponin-T test (NPV) was 76.2%.

Table 19. Distribution of sample population according to ECG changes with troponin T test.

ECG	Troponin-T n (%)		Total n (%)
	Positive	Negative	
ECG changes	21 (84%)	4 (16%)	25 (100%)
No ECG changes	8 (32%)	17 (68%)	25 (100%)
Total	29 (58%)	21 (42%)	50 (100%)

Pearson Chi-Square: 13.875

P value (significant at 0.05 level): < 0.001

Odds ratio – 11.156

95% confidence interval @ 2.864 - 43.46

There is significant association between ECG changes and a positive Troponin T test.

Table 20. Correlation between ECHO changes and troponin T test.

ECHO	Troponin-T n (%)		Total n (%)
	Positive	Negative	
Abnormal	20 (95.2%)	1 (4.8%)	21 (100%)
Normal	9 (31%)	20 (69%)	29 (100%)
Total	29 (58%)	21 (42%)	50 (100%)

Pearson Chi-Square : 20.611

P value (significant at 0.05 level): < 0.001

Odds ratio – 44.44

95% confidence interval @ 5.141 – 384.210

Significant association between ECHO changes and Troponin T test is seen in our study.

Table 21: Correlation between Ejection fraction and troponin T card test.

Troponin T test	N	Mean	Std. Deviation
Negative	21	66.29	3.452
Positive	29	53.41	10.480
Total	50	58.82	10.431

- **Student t test** was done to compare means between 2 outcome groups.
- Mean difference - 12.87
- ‘ t ‘ statistic – 5.407
- P value (significant at 0.05 level) - <0.001
- 95% confidence interval of the difference @ 8.08 – 17.65

The difference between the mean ejection fraction between the infants with positive troponin-T test and negative troponin-T test was statistically significant.

DISCUSSION

Cardiovascular dysfunction is one of the commonest complications in infants with perinatal birth asphyxia. The sequelae following birth asphyxia pertaining to cardio-vascular system ranges from transient myocardial ischemia, valvular insufficiency, decreased left ventricular contractility and output to pulmonary hypertension and systemic hypotension.

This study was done to determine these cardio-vascular changes resulting from asphyxia and to evaluate various diagnostic modalities in early diagnosis of myocardial injury, thereby facilitating early and better management and subsequent reduction in morbidity and mortality in these asphyxiated infants. In this study, we compared the elevation of Troponin-T with parameters like ECG changes involving T wave, ST segment and Q wave and echocardiographic features like valvular regurgitation, ejection fraction, shunts and pulmonary hypertension for their relationship with severity of asphyxia and outcome of the cases

TABLE 22: Comparison with other studies

Parameter	Our study	Kanik et al[13]	Costa et al[14]	Rajakumar et al[15]	Kilic et al[16]	Marta et al[24]
Sample	50 asphyxiated	34 asphyxiated	29 asphyxiated 30 controls	30 asphyxiated 30 controls	30 asphyxiated 30 controls	39 asphyxiated 44 controls
ECG Changes	Present in 25 infants	Present in 13 infants	Present	Present in 17 cases		
Echo changes	Present in 21cases (18 were TR)	Present in 1 infant	Present	Present in 7 cases		Present
Ejection fraction	Reduced and correlates with severity & outcome		Reduced in asphyxiated	Reduced		
Troponin-T	Elevated and correlates with severity & outcome	Troponin-I Elevated (levels high in non-survivors)	Troponin-T elevated in asphyxiated	mean serum levels of troponin-T high in cases	higher TnT levels in cord blood & venous blood of cases than controls	mean serum levels of troponin-T high in cases
Mortality	11 infants	9 infants		9 cases (30%)	11 cases	
Remarks	Troponin-T is highly sensitive for early diagnosis of myocardial injury in asphyxiated	Troponin-I & CK-MB were studied no predictive value for CK-MB.	Troponin-T valuable tool for myocardial injury in asphyxiated	Troponin-T had better sensitivity and specificity than CK-MB and correlated with outcome	CK-MB levels in venous and cord blood were also elevated in the study group	Troponin-T valuable tool for myocardial injury in asphyxiated

TABLE 22 Continued…

Parameter	Our study	Agrawal et al[18]	Matter et al[19]	Boo et al[22]	Clark et al[23]	Guenes et al[17]
Sample	50 asphyxiated	60 asphyxiated	25 asphyxiated 20 controls	50 asphyxiated 50 controls	47 in PICU 60 controls	45 asphyxiated 15 controls
ECG Changes	Present in 25 infants	Present in 46 infant	Present			
Echo changes	Present in 21cases (18 were TR)		15(TR) in cases & 7(TR) in controls High Tei index in cases			present in 12 cases
Ejection fraction	Reduced & correlates with severity, outcome		Reduced in asphyxiated	Reduced		
Troponin-T	Elevated and correlates with severity & outcome	CK-total, CK-MB, Troponin-I Elevated (levels high in non-survivors)	mean serum levels of troponin-T high in cases	higher TnT & CK-MB levels in cord blood of cases than controls	troponin-T levels high in PICU infants (esp. in <1 month of age)	mean serum levels of troponin-T high in severe asphyxia
Mortality	11 infants	16 infants	6 infants	11 cases	2 cases	
Remarks	cTnT is highly sensitive for early diagnosis of myocardial injury in asphyxiated	Troponin-I & CK-MB were elevated in asphyxiated and in non-survivors	Troponin-T levels correlated with Tei index. Doppler tissue imaging (DTI) has high sensitivity than echo.	Infants with CCF within 48 hrs,infants with EF <60% ,non-survivors had high cTnT not CK-MB	Positive correlation between cTnT and mortality score	cTnT valuable tool for myocardial injury in asphyxia than CK-MB

The role of cardiac enzymes in detection of ischemic damage to heart is well established in adults. But recently, their role in detection of ischemia in newborns due to perinatal birth asphyxia has been under debate. Initially, CK-MB and troponin-I were studied for their diagnostic role in asphyxiated newborns. Kanik et al[13] showed that troponin-I had better sensitivity than CK-MB and the study also concluded that there is no predictive value for CK-MB. Agrawal et al[18] also compared troponin-I and CK-MB and got similar results, claiming the superiority of troponin-I over CK-MB in diagnosis of myocardial damage in newborns with birth asphyxia. Of later, cardiac troponin-T has been under investigation for the same purpose.

Costa et al[14] and marta et al[24] proved the elevation of cardiac troponin-T was significant in asphyxiated newborns. Guenes et al17 re-inforced that troponin-T elevated to a greater extent in severe asphyxia than mild asphyxia and cardiac troponin-T had much better sensitivity than CK-MB. Many comparative studies were done to investigate cardiac troponin-T against CK-MB for diagnostic significance in asphyxiated newborns. Rajakumar et al[15] and Boo et al[22] reproduced the better results for troponin-T in comparison with CK-MB.

Clark et al[23] also showed that cardiac troponin-T levels in the serum correlated with the severity of asphyxia and paediatric mortality score. Based on these researches of late, a single use card test was designed to detect the elevation of troponin-T in serum. While most of the above mentioned studies investigated serial measurements of troponin-T, this study investigated a simple card test done in anti-coagulated venous blood, which has the advantage of bedside testing and much quicker results. The test was done on Day 1 between 12-24 hrs as cardiac troponin-T tends to rise in this time-limit. The TROPONIN T card test is designed to yield a positive result for cardiac Troponin concentrations $\geq$ 0.08 ng/ml. The efficacy of this test in terms of sensitivity and specificity was evaluated in comparison to clinical diagnosis of myocardial injury.

The study population included 29 males and 21 females, who were examined for congenital heart disease prior to their inclusion in the study. Only neonates with Apgar score at 1 minute less than 6 were included. Among the 50 neonates, 22 neonates had severe asphyxia (Apgar 1-3) and 28 neonates had moderate asphyxia (Apgar 4-6). So all these neonates needed some kind of resuscitation and were given accordingly. Resuscitation using bag and mask was given to 40% of the neonates while 44% needed bag and tube for resuscitation.

According to Sarnat and sarnat classification, 6 infants were in HIE stage I, while 25 and 19 neonates were in stage II and III, respectively. A 12 lead ECG was taken in all the 50 infants to determine the ischemic changes due to asphyxia. A normal ECG in a neonate depicts the right ventricular dominance due to the hemodynamic relationship in utero. Thus ECG in neonates is expected to evolve over the days in neonatal period due to the physiological changes. All these physiological changes were taken into account in detecting the ischemic changes pertaining to asphyxia.

About 50% of the neonates had one or more ECG changes pertaining to ischemia according to Jedeikin et al[9]. T wave changes were measured in all leads except for aVR, as it is physiological to have T wave inversion in aVR. 17 neonates had flat or inverted T waves in 1 or 2 leads, while 8 neonates had these changes in more than 2 leads. ST segment depression or elevation in atleast 2 standard leads of magnitude more than 1 millimetre was considered significant. These changes were not found in any of the neonates studied. According to Farru o et al[25], Q wave changes are rare and is present indicate severe form of myocardial ischemia. There was no Q wave changes detected in the study population which may be due to a small sample size in the study.

There was a statistically significant association between these ECG changes and HIE staging. As the severity of asphyxia increased according to the HIE staging, the prevalence of ECG changes also increased in the sample population. Furthermore, neonates who had ECG changes have 4 times more risk of having myocardial injury clinically when compared to neonates with no ECG changes. This indicates that ECG changes can be used to predict the development of clinical signs and symptoms of myocardial injury. The ECG changes also correlated with the outcome of the neonates, as the neonates with ECG changes were more prone for worse outcome than neonates with no ECG changes. These results are similar to one obtained by Agrawal et al[18] and Rajakumar et al[15], reinforcing the fact that a simple bedside ECG can be used as a determinant of myocardial injury and as a predictor of mortality.

In terms of sensitivity and specificity, ECG was much inferior to echocardiogram and troponin-T card test with a positive predictive value of 80% and negative predictive value of only 52%. This low negative predictive value reflects that a normal ECG does not rule out a potential underlying myocardial ischemia. Although it has good positive predictive value and sensitivity of 62%, ECG cannot be used as a solitary diagnostic modality for early detection of myocardial injury.

An echocardiogram was performed on all infants under the study, on their 2nd day of life to detect the cardiac abnormalities due to asphyxia. Although 29 infants had a normal echocardiogram, it was abnormal in the remaining 21 infants reflecting the structural changes due to myocardial damage. Tricuspid regurgitation was the commonest if these abnormalities found in 18 infants. It occurs due to ischemic damage of tricuspid valve papillary muscle and pulmonary hypertension as a result of the right ventricular myocardial disease. Presence of tricuspid regurgitation also correlated with the severity of asphyxia, as it was more common among infants with HIE stage 3. Pulmonary hypertension alone was found in 1 infant while 2 infants had a combination of pulmonary hypertension and tricuspid regurgitation. All these 3 infants with solitary pulmonary hypertension or in combination were in stage III of HIE staging, reflecting that it is more common among the infants with severe asphyxia. Mitral incompetence was not observed in any of the infants. Furthermore Infants who had echocardiographic changes pertaining to perinatal asphyxia have 28 times more risk of having myocardial injury clinically when compared to infants with normal echocardiographic changes.

Echocardiographic changes also were significantly associated with the outcome, as infants with these changes were at more risk of having a

worse outcome. When compared to ECG, echocardiogram was far better in terms of specificity and predictive values in diagnosing the myocardial injury which manifests clinically but in terms of sensitivity (62.5%), it was at par with ECG. In fact, the specificity of echocardiogram (94.4%) was higher than troponin-T card test (88.9%). This reflects the importance of screening all the asphyxiated infants for structural myocardial damage facilitating early diagnosis and management, in turn improving the outcome of these infants.

Regarding the troponin-T levels in infants with echocardiographic changes, these infants had 44 times more risk of testing positive in the card test. So, it can be said that almost everyone who has significant structural ischemic changes in echocardiogram are expected to test positive for elevation in troponin-T. No regional wall motion abnormalities and shunts were observed in the study population. Overall, echocardiographic changes have good specificity but lack of sensitivity reinforces the need for other sensitive bedside test like troponin-T card test.

Left ventricular ejection fraction was measured for all the infants, as cardiac output was expected to be diminished due to asphyxia. There was a negative linear correlation between ejection fraction and HIE staging (severity of asphyxia) i.e., ejection fraction decreased with the

increasing severity of asphyxia. The difference between mean ejection fraction of infants in stage 1 and stage 2 HIE was not significant statistically but it was significant, when comparing with stage 3 HIE. There was also a significant association between the levels of ejection fraction and occurrence of worse outcome. So, ejection fraction can be used for quantitative assessment of severity of ischemic injury and as a predictor of mortality. Moreover, mean ejection fraction in infants with a positive troponin-T test was much lower (53%) than the infants with a negative troponin-T test (66%). So, we can conclude that troponin-T test is also a indirect measure for reduction in ejection fraction in the asphyxiated infants. These findings regarding ejection fraction and its relationship with severity of asphyxia and outcome in this study correlates with the similar findings observed in Costa et al[14], Rajakumar et al[15], and Matter et al[19].

The simple bedside cardiac troponin-T card test done in venous blood which was investigated in this study was positive in 29 of the 50 infants indicating the level of troponin-T in these infants are higher than 0.08 ng/ml. About 17% and 40% of the infants in HIE stage 1 and 2 respectively, tested positive for troponin-T card test. But, about 95% of the infants in stage 3 of HIE staging tested positive in troponin-T card

test. These figures depict that likelihood of the test being positive increases with increase in severity of asphyxia.

About 38% of the infants who tested positive had the worse outcome of death while all infants with a negative test survived. So, it is obvious that infants with positive troponin-T card test are at more risk of dying than infants with a negative troponin-T card test. Among all the parameters studied, troponin-T elevation as evidenced by a positive card test is the best predictor of mortality. This fact coincides with the findings in other studies like Boo et al[22] and Clark et al [23].

Moreover infants with abnormal ECG changes and echocardiographic changes had 11 times and 44 times more risk of having a positive troponin-T card test, respectively. As already stated, the mean ejection fraction in infants with a positive troponin-T test was much lower (53%) than the infants with a negative troponin-T test (66%). Thus, troponin-T elevation coincides well with occurrence of abnormal ECG, echocardiographic findings and reduction in ejection fraction due ischemia.

In terms of sensitivity, troponin T has a better sensitivity (84.4%) and when compared to ECG and echocardiogram, as both of them had a low sensitivity of only 62%. In terms of specificity, troponin T has a better specificity (88.9%) when compared to ECG (72%) but

echocardiogram had higher specificity (94.4%) than troponin-T card test. Ideally a screening test should have very high sensitivity and specificity. But in clinical terms, it is more important to have a test with high sensitivity at an acceptable level of specificity (above 80%).

In spite of having high specificity, echocardiogram lacks the much needed sensitivity, diminishing its role as a screening test for myocardial injury in asphyxiated infants. Regarding predictive values, troponin-T test had a much better negative predictive value (probability of not having the clinical disease, when the test is negative) than ECG and echocardiogram. Thus, chance of having developing clinical signs and symptoms of myocardial injury after a negative troponin-T card test is very low. The positive predictive value (probability of having the clinical disease, when the test is positive) was also high (93%) at par with echocardiogram (95%) and much better than ECG (80%).

Besides the current study, superiority of troponin-T as a diagnostic test of myocardial injury in asphyxiated infants was also observed in many studies. Costa et al[14] observed that Troponin-T levels were elevated in asphyxiated infants while Rajakumar et al[15] observed that Troponin-T had better sensitivity and specificity than CK-MB and it also correlated with outcome. Kilic et al[16] found higher cardiac troponin-T levels in cord blood & venous blood of cases than controls.

In our study population 15 infants (30%) who had clinical evidence myocardial injury tested positive for all the three diagnostic modalities ECG , ECHO, Troponin T .

Our study findings regarding sensitivity of troponin-T correlates well with findings of Matter et al[19] (Troponin-T levels correlated with Tei index and ejection fraction. Doppler tissue imaging (DTI) has high sensitivity than echo) and Boo et al[22] (Infants with congestive cardiac failure within 48 hrs of life, infants with EF <60%, non-survivors had high cardiac troponin-T levels but not CK-MB). The correlation between troponin-T and mortality observed in the study coincides with one observed by Clark et al[23] (Positive correlation between cTnT and paediatric infant mortality score).

Overall, it can be concluded that bedside troponin-T card test has high sensitivity and specificity in comparison to ECG and echo and it is an invaluable tool for early detection of myocardial injury due to perinatal birth asphyxia. However, much larger controlled trials are needed to recommend this test as a marker for myocardial damage in perinatal asphyxia.

LIMITATIONS OF THE STUDY

a. There was no control group included in the study
b. Quantification of Troponin-T levels was not possible as we used a rapid diagnostic card test.
c. Comparison with other cardiac bio-markers like CK-MB, LDH, troponin-I was not done
d. Autopsy was not done in the non-survivors.

CONCLUSION

Perinatal Asphyxia is a multi system disorder. Most of our recent therapeutic strategies are directed towards revitalizing the brain. The cardiac complication of perinatal asphyxia takes a back stage in our current treatment protocols. Non availability of a simple, reliable bedside test to diagnose myocardial injury is the reason behind this.

Our study aims to bridge this gap by providing a cardiac Troponin T card test for diagnosing myocardial ischemia.

Although many studies are available for estimating the serum level of Troponin T in asphyxiated neonates, there is no study regarding the Troponin T card test. So our study is unique and first of its kind in utilizing Troponin T card test in asphyxiated neonates. In our study we found that Trop Tcard test has a positive predictive value of 93.1% and negative predictive value of 76% which frames this test an ideal screening tool for early diagnosis of myocardial injury.

In resource limited setting where the accessibility to 12 lead ECG, ECHO and aid of cardiologist are not available , Trop T card test will serve a valuable handy screening tool.

We would like to conclude that Troponin T card test is a valuable tool for early detection of myocardial injury due to perinatal birth asphyxia and further large trials are needed in the future to validate it as a standard diagnostic tool in managing asphyxiated NICU neonates.

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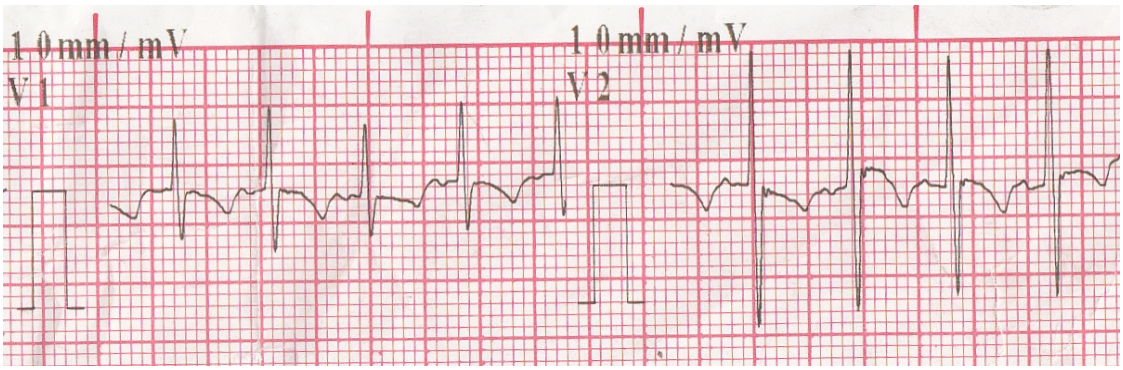
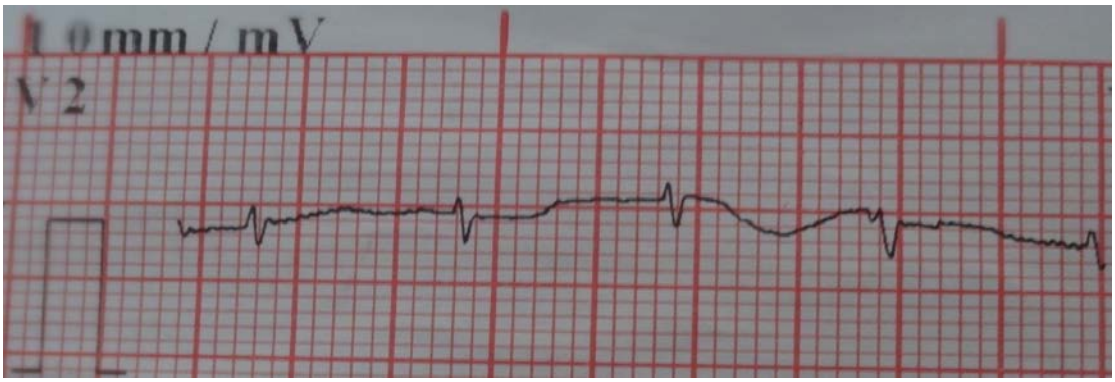
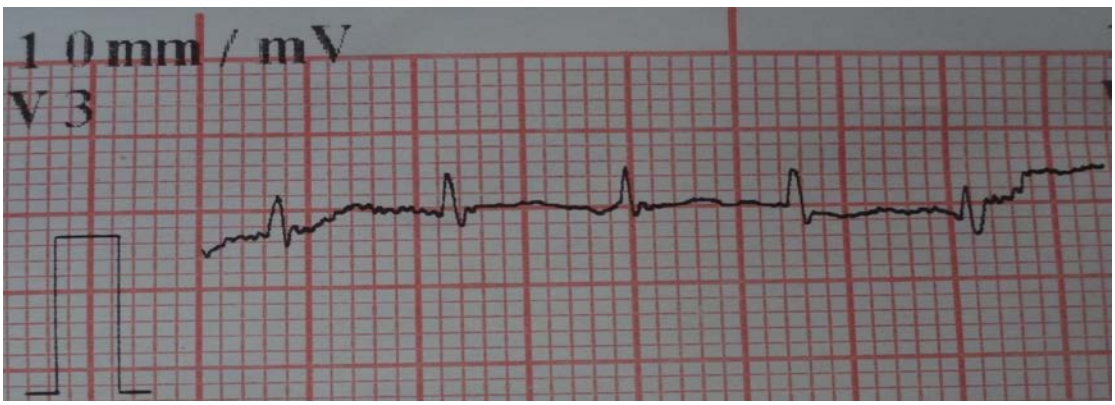
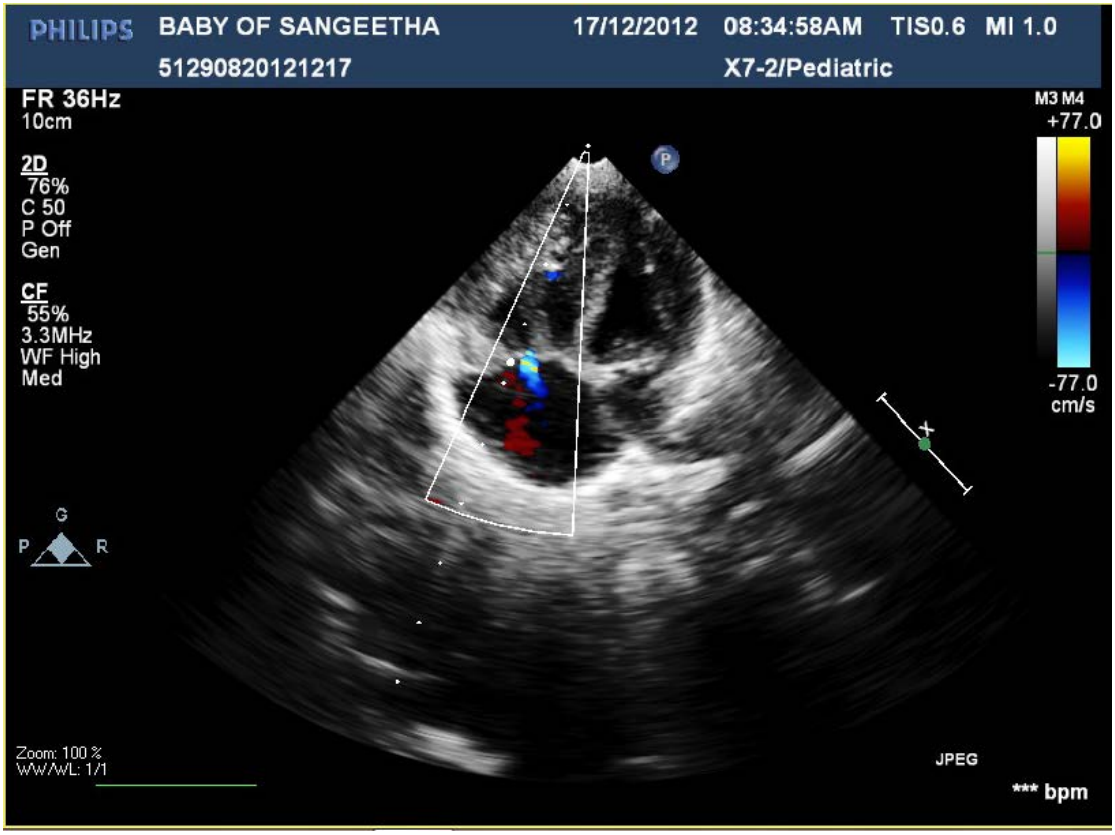
ECG SHOWING INVERTED AND FLAT T WAVES



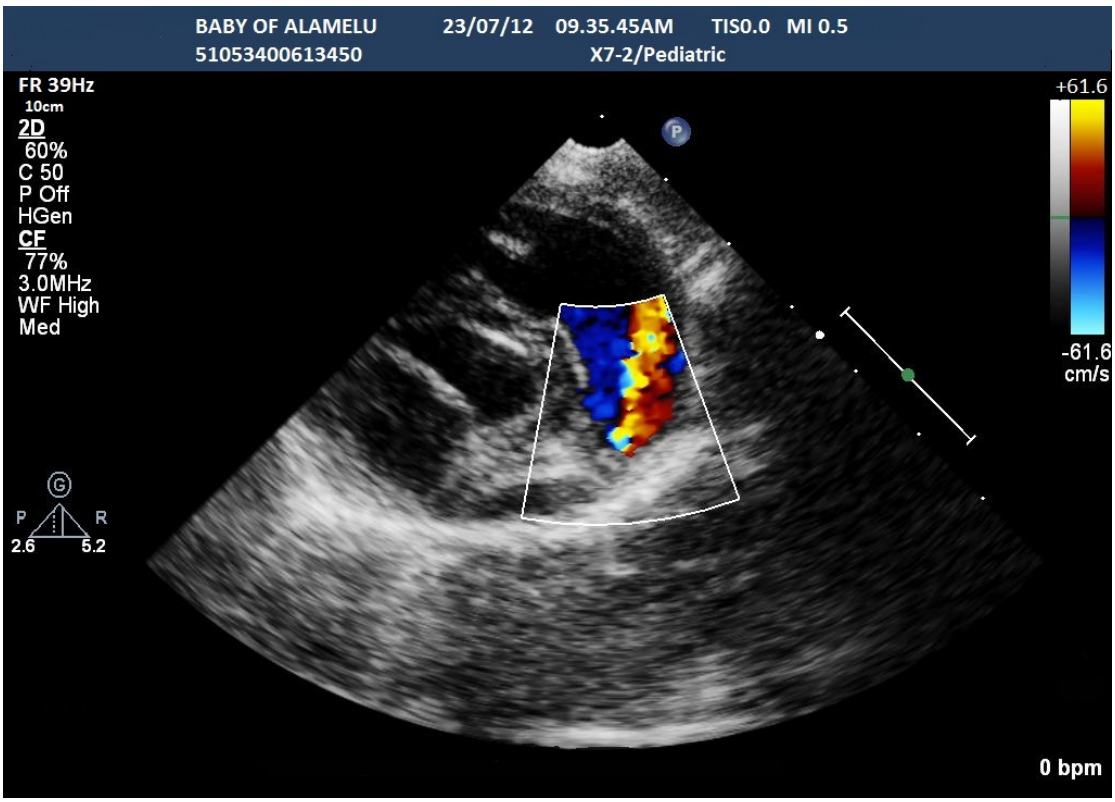




ECHO SHOWING TRICUSPID REGURGITATION

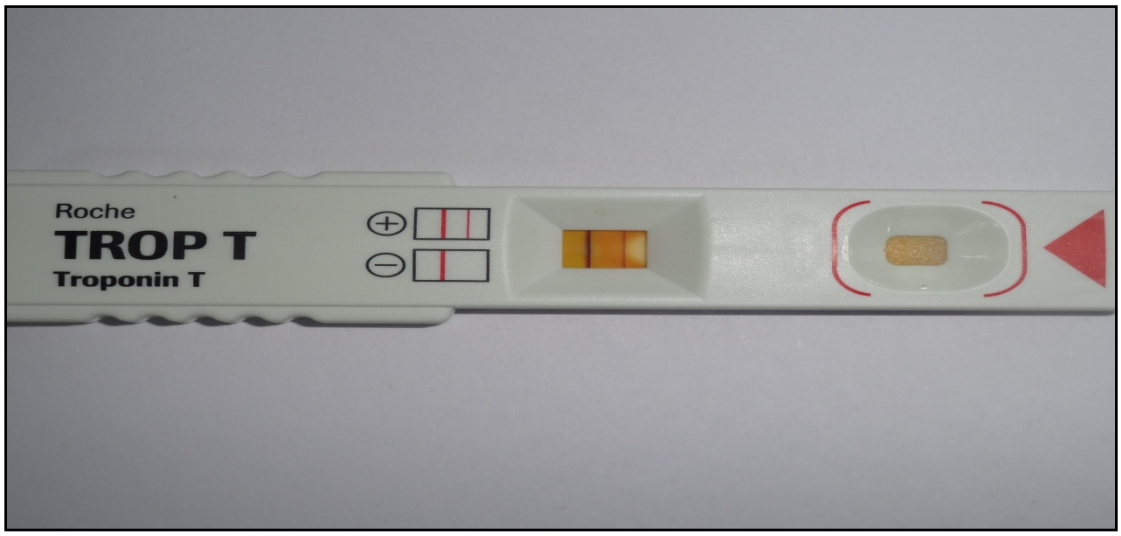


ECHO SHOWING PULMONARY HYPERTENSION



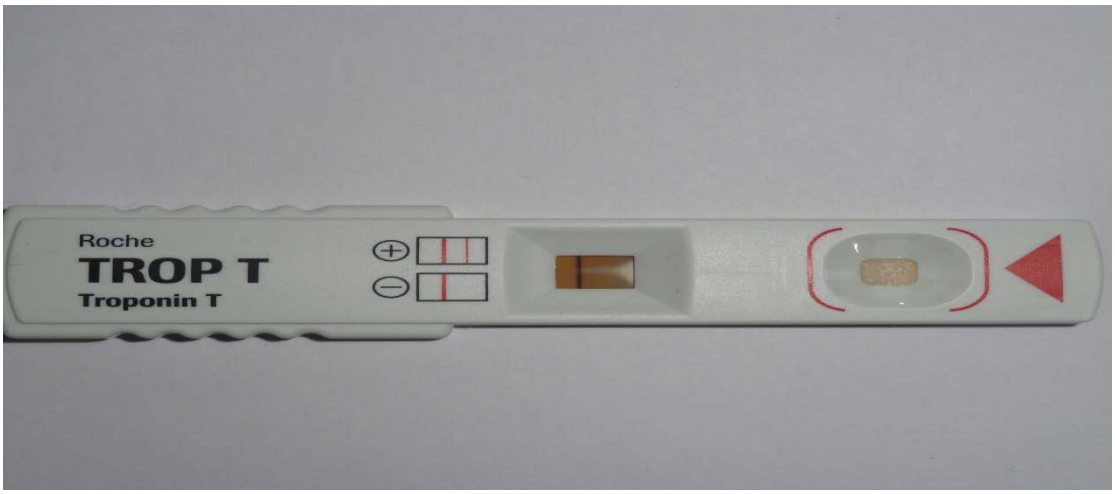
TROPONIN T CARD TEST RESULTS

POSITIVE TEST



Two lines seen in the read window indicates positive results

NEGATIVE TEST



Single line seen in the read window indicates negative results